ESSENTIAL GUIDES FOR ISOLATION/ PURIFICATION OF NUCLEIC ACIDS

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Introduction

Isolation/purification of nucleic acids (NAs – dsDNA, ssDNA, tRNA, mRNA, etc.) is a common and crucial step in most molecular biology, biomolecule engineering, cancer research, recombinant DNA, forensic analysis, gene therapy, DNA vaccines and diagnostics (e.g. DNA chips) applications. Most often, NAs are isolated and purified merely as a way of obtaining quantitative and qualitative information about a certain sample (e.g. paternity testing, screening for viruses in clinical samples, etc.). Several purification methods have been developed by laboratories and companies involved in the above-mentioned areas. These protocols and processes are based on the same general principles, and follow three main stages: obtaining the NA source (cell growth, tissue isolation, chemical or enzymatic reaction), primary isolation and purification. Different techniques exist that can be used alone or in combination within each step of the isolation/purification scheme (**Figure 1**).

When choosing an isolation/purification protocol or process, NA researchers and manufacturers should take into account several aspects. Especially important are the nature of the target NA, the final application, cost and availability of the technique (**Table 1**). The final application will determine which specifications (yield, purity, safety, efficacy, identity) the final NA preparation should follow.

Nucleic Acids

The success of NA purification relies on a minimal understanding of the molecular composition and structure of the target molecule. NAs are linear polymers of nucleotides (adenylate, guanylate, cytidylate and uridylate in RNA and deoxyadenylate, deoxyguanylate, deoxycytidylate and thymidylate in DNA) linked by phosphodiester bonds. The presence of negatively charged phosphate groups in the backbone of the molecule confers a polyanionic

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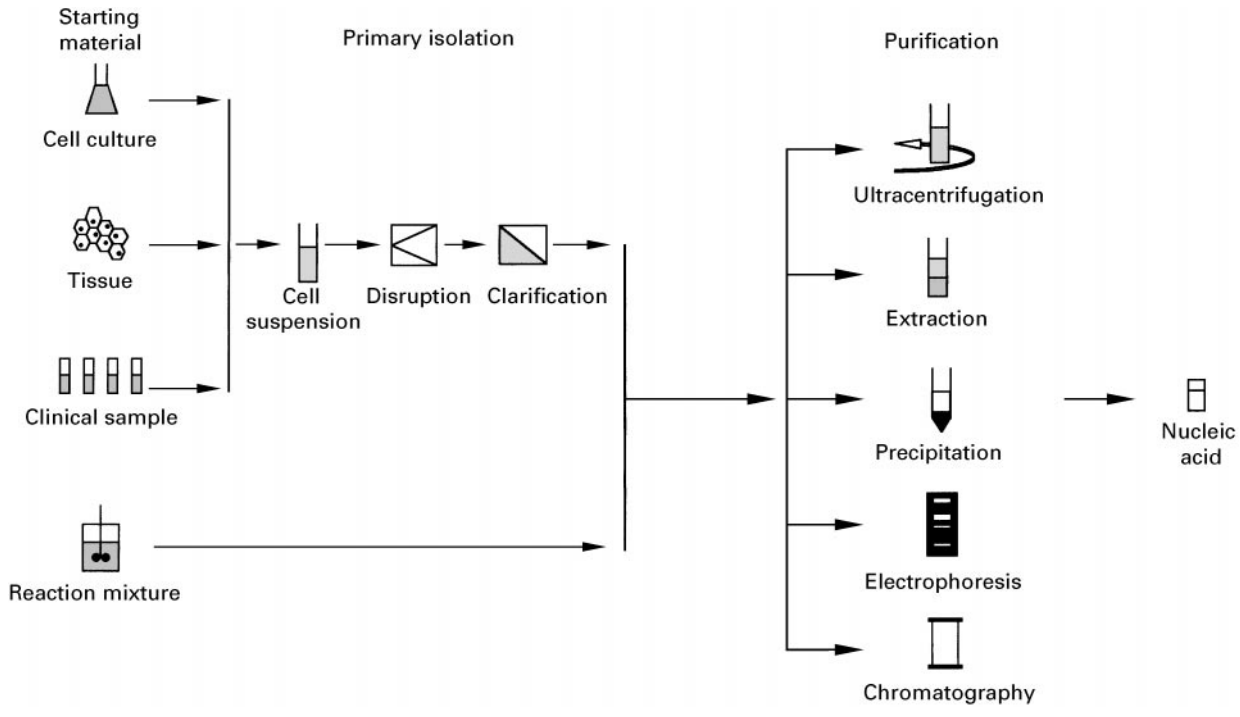

Figure 1 Strategies for the isolation/purification of nucleic acids.

Table 1 Relevant aspects to the choice of a nucleic acid isolation/purification protocol or process

Target nucleic acid
DNA or RNA
Size (from a few base pairs to 100 kb or more)
Base composition (e.g. poly(A)$^+$ segments)
Structure (single/double strand, secondary and tertiary structure, supercoiling)
Application
Material for research (cloning, sequencing, *in vitro* translation, etc.)
Material for therapeutic use (gene therapy, gene marking, DNA vaccines, antisense)
Material for identification and quantification (diagnostics, forensics, medicine)
Specifications
Purity
Yield
Potency
Safety
Identity
Source
Cells
Prokaryotic (bacteria)
Eukaryotic (plant, animal, fungi including yeast)
Viruses (M13, phage λ, human immunodeficiency virus, hepatitis)
Chemical and enzymatic mixture
Oligonucleotides from solid-phase synthesis
Restriction digests
Polymerase chain reaction mixtures
Labelling and modifying reaction mixtures
Type of processing
Single sample
High-throughput
Parallel, *n* samples with the same target NA
Parallel, *n* samples with different target NA
Sequential, *n* samples with same target NA
Sequential, *n* samples with different target NA
Cost
Per number of isolation (diagnostics)
Per amount of target DNA (large scale manufacture)
Other
Time
Scaleability of process
Environmental issues
Safety of protocol
Robustness
Automation

nature to NAs. DNA molecules are often double-helix structures formed by two strands winding around each other and around a common axis. These structures are stabilized by hydrogen bonds and mainly by stacking forces. The helix axis can also be coiled, forming a higher order structure, named supercoiling. RNA can take on the same configurations as DNA, having secondary and tertiary structures; it can be single-stranded (more often) or double-stranded, linear (more often) or circular, and it can also form hybrid helices with DNA. Double-stranded NAs melt to single strands fairly sharply as condition (temperature, OH^-, denaturants like formaldehyde or formamide) becomes more denaturing. Renaturation is achieved when the reversible denaturation condition is removed. However, if the denaturation condition is quickly removed, the process is irreversible. The stability of double strands increases with the mole fraction of GC pairs and decreases as the pH is varied towards either side of neutrality. RNA is particularly sensitive to alkaline conditions.

Primary Isolation

Cell Disruption

Cells from the source organism are first recovered from the starting material, whether it is a cell culture, tissue or clinical sample, and resuspended in an appropriate buffer. The isolation of the target NA then starts with the disruption or lysis of the cells. There are several cell disruption methods that can be used alone or in combination (**Table 2**).

The choice of disruption method should consider factors such as the effect on the final NA, the cell type (animal or plant culture or tissues; fungi or Gram-negative or -positive bacteria spheres or filaments; viruses), the scale (laboratory- or industrial-scale), sample volume and number of samples, associated costs, duration and final application.

Usually, cell disruption is combined with a chemical or an enzymatic step for the inactivation of intracellular nucleases, which can degrade the target NA if a controlled method is not properly adopted. The physical and chemical conditions present during cell lysis are a key step in NA isolation. Temperature should be kept around 4°C to avoid nuclease activities that degrade the NAs. When an enzymatic step is included (performed at around 25–35°C), for instance by adding proteinase K or RNase to hydrolyse proteins or contaminating RNAs, caution should be taken with the presence of endogenous DNases that are active in the same temperature range.

The release of NAs during lysis significantly increases the viscosity of the solution, making mixing a difficult task. The lysates show non-Newtonian properties, exhibiting a rheological behaviour that makes flow and handling of the material very difficult. This issue is particularly troublesome on a large scale. The mixing at this stage should be very gentle to avoid shearing of the target NA. In certain protocols and processes it is important to maintain the contaminating NAs with the highest molecular weight possible, in order to facilitate their removal in the subsequent steps. Glucose or sucrose is often included in the lysis buffer or after disruption, in order to protect NAs against shearing. Sensitivity to shear increases with molecular weight, and a single-stranded NA is more sensitive than a double-stranded one with the same length. Special care should thus be taken when handling (pipetting, pouring, mixing) NA solutions of high molecular weight.

The lysis buffer usually contains a chelating agent such as ethylenediaminetetraacetic acid (EDTA). The removal of divalent cations (mainly Ca^{2+} and Mg^{2+}) from biological membranes and cell walls (when present) destabilizes their structure, facilitating lysis, and reduces the activity of endogenous Mg^{2+}-dependent nucleases, preventing NA degradation. Protein-denaturing agents (e.g. detergents, phenol, guanidinium hydrochloride) are commonly added. The detergents denature proteins, including the ones associated with NAs, and solubilize lipids from membranes, facilitating cell disruption. Finally, the lysis buffer pH must be tightly controlled and local pH extremes avoided, since low pH values promote the hydrolysis of DNA, and alkaline pH favours the cleavage of RNA and the irreversible denaturation of chromosomal DNA. Moreover, very low pH values lead to depyrimidation and depurination.

RNA is notoriously susceptible to degradation and special care is required in its purification. The presence of endogenous and/or exogenous RNases is a major concern, since RNases can recover activity even after harsh denaturation treatments (e.g. boiling). High concentrations of strong chaotropic agents (e.g. guanidinium hydrochloride, guanidinium thiocyanate, caesium trifluoroacetate) is often used to inactivate RNases irreversibly and simultaneously promote the disruption of cellular membranes.

Table 2 Summary of cell disruption methods

Physical	*Chemical*	*Biological*
Pressure	Detergents	Enzymes
Ultrasoud	SDS	RNase
Blades	Triton	Lysozyme
Grinding	Acid	Proteinase K
Freeze–thaw	Alkali	Viruses
Osmotic shock	Organic solvents	
Dehydration	Phenol	
	Chaotropic salts	
	Urea	
	Guanidinium hydrochloride	
	Guanidinium thiocyanate	
	Caesium trifluoroacetate	
	Thiol reduction	

Clarification of Lysates

After cell lysis and inactivation of endogenous nucleases, cellular debris, proteins and other precipitated molecules must be removed. This is usually achieved by means of centrifugation or filtration steps. During this operation, certain amounts of NAs can be lost in the liquid entrained in the solid phase. Extensive removal of water from the solid phase can be performed to increase the yield, but this operation usually also increases the amount of impurities. Minimum shear should be exerted in order to preserve the integrity of the target NA. The solution obtained after clarification is more or less rich in several impurities. Small molecules such as nucleotides, nucleosides,

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amino acids, sugars and inorganic ions are easily removed, but DNA, RNA, polysaccharides and proteins are difficult to remove because these macromolecules have some common physical and chemical characteristics. The impurity profile and amount in a clarified lysate depend not only on the cell type, but also on factors such as phase growth and growth conditions (carbon and nitrogen sources, media richness, dissolved CO_2 and O_2, pH, etc), as well as on the method used for disruption (Table 2).

Purification

After the primary isolation steps, most impurities in solution are comprised of NAs from the source organism, denatured forms of the target NA, proteins and endotoxins (Gram-negative bacteria). The similarities between these molecules and their wide molecular weight range make purification difficult. Several methods are available to purify NAs, depending on the amount, yield and purity needed, and also on the availability of methods and associated costs.

Gradient Centrifugation

A classical method for separating DNA or RNA from each other, and from proteins and polysaccharides, uses equilibrium-density gradient (or isopycnic) centrifugation, which is based on differences in particle density. The density gradients can be self-forming or pre-formed continuous gradients, prepared with caesium salts (e.g. CsCl, Cs_2SO_4) or iodinated compounds (e.g. metrizoate, metrizamide, nycodenz). Equilibrium-density gradient centrifugation is one of the most efficient methods available for the purification of NAs. The major drawbacks are its dependence on high cost reagents and equipment (the ultracentrifuge) and the fact that it is a time-consuming operation (typically at least 48 h of centrifugation). When using this method, it should be kept in mind that centrifugal conditions may affect the native characteristics of the sample. For instance, the presence of endogenous and/or exogenous nucleases during a long-term process can lead to NA degradation. In addition, the chemical constituents of the solutions can affect the integrity of NAs. Physical degradation as a result of shear stress should be avoided, too.

The position of NAs in the gradient is usually located by measuring the absorbance at 260 nm. However, in a CsCl isopycnic separation, the DNA molecules band at approximately the same density. An alternative approach in this case is to band DNA in CsCl gradients in the presence of ethidium bromide or propidium iodide, which intercalate differentially between the bases of DNA, resulting in different molecule buoyant densities. This technique allows the separation of supercoiled plasmids, nicked or relaxed plasmid forms, genomic DNA, RNA and proteins.

Liquid–Liquid Extraction

Solvent extraction is often used to remove proteins and lipids from NAs. The pH values of the extractant organic phase and of the starting aqueous phase are very important because partition coefficients of NAs are pH-dependent. A classical system for solvent extraction uses a sequential extraction with phenol : chloroform (1 : 1). Although phenol is an efficient denaturant of proteins, it does not completely inactivate RNases, and it solubilizes RNA with long poly(A)$^+$ tails. These problems can be partially overcome by introducing isoamyl alcohol in the phenol/chloroform mixture.

In spite of the efficiency of solvent extraction, residual contaminants still remain in solution. Furthermore, solvents like phenol and chloroform are extremely toxic for both the operator and the environment. Even small amounts in the final NA preparation are potentially toxic to live recipient cells and can interfere in downstream experiments. This makes solvent extraction an unacceptable method when preparing NA for therapeutic use.

Extraction of NAs has also been performed with aqueous two-phase systems. This technique relies on the fact that concentrated aqueous solutions of polysaccharides, such as dextran and polyethylene glycol (PEG), are immiscible. Many biological components (polymers, cells, cell organelles) including NAs show different solubility in the two phases formed, and will therefore separate by partitioning. By manipulating the system conditions (e.g. buffer, polymer and salt concentration) it may be possible to separate DNA from RNA, native from denatured DNA and single from double or triple strands. The technique, however, has not been studied in depth, and therefore is not commonly used.

Precipitation

Precipitation with ethanol or isopropanol is commonly used to concentrate NAs. Typically, 2 vol of ethanol or 0.7 (v/v) isopropanol is added to the NA solution. The process is more efficient if performed in the presence of moderate concentrations of monovalent cations and at low temperatures (below 4°C). The concentration and type of salt (cation: ammonium, lithium, sodium, potassium; anion; acetate, chloride) used in precipitation should be optimized for the target NA. The duration and speed of the centrifugation step used to recover the precipitate

material are also important, and should be adjusted according to the concentration of NA (longer and faster centrifugation for lower concentrations). If the amount of target NA is low, an inert carrier such as glycogen can be added to the mixture to increase precipitation efficiency. After draining liquid from the precipitated material, an appropriate buffer is added to redissolve the NAs.

Another method for precipitation of NA uses PEG/salt (NaCl, $MgCl_2$) systems. The method is based on the fact that the size of the DNA molecule precipitated by PEG is dependent on the concentration and molecular weight of PEG. It can thus be used either to fractionate DNA according to molecular mass, or simply to precipitate the total DNA content. The method is rapid and inexpensive but consistent yields are difficult to obtain.

The precipitation methodology, although generally used to concentrate NAs, is also effective as a purification step. Selective precipitation could be achieved, for instance, with high concentrations of different salts or changes in pH to precipitate proteins or nontarget DNA or RNA. For example, ammonium sulfate is often used to precipitate contaminating proteins. Lithium chloride precipitation has also been used in the preparation of large RNA. It takes advantage of the fact that small RNAs (tRNAs and 5S RNA) are soluble in solutions of high ionic strength, whereas large RNAs (rRNAs and mRNAs) are insoluble and precipitate out. After centrifugation, the high molecular weight RNA is redissolved in water or buffer.

Gel Electrophoresis

Gel electrophoresis on agarose or polyacrylamide gels is a powerful technique commonly used to separate, identify and purify NAs. Under the effect of an electrical field, NA molecules migrate through the gel matrix at a rate that is inversely proportional to their size. The resolving power of gel electrophoresis is extremely high, enabling the separation of molecules that differ in size by as little as 1 bp. The position of the individual molecules in the gel can be determined by staining with a fluorescent intercalating dye such as ethidium bromide. By using a number of techniques, such as electroelution or low-melting agarose gels, it is possible to recover DNA of high purity from the gels. However, the scale associated with electrophoresis is generally small.

Chromatography

Chromatography has been increasingly used to purify NAs. The technique is powerful, rapid and simple to perform, while avoiding the use of toxic compounds common to competing technologies like ultracentrifugation and phenol/chloroform extraction.

Disposable column cartridges packed with particles of a stationary phase, and operating in the gravity flow format, often constitute an essential part of commercial isolation kits for lab-scale applications. In many cases, centrifugation is combined with disposable spin columns to enhance and speed the isolation. High-throughput and automated formats using these column cartridges are increasingly available that enable the processing of several samples at the same time, minimize hands-on preparation time, and reduce the risk of sample mix-ups. Purification using high pressure liquid chromatography (HPLC) has also been performed as a preparative step to isolate small quantities of NAs. In large scale applications, column chromatography is a central process step. For large scale applications, economic constraints demand regeneration and re-use of the expensive chromatographic media. Furthermore, if material is being produced for clinical use, validation of the cleaning is indispensable.

Different types of chromatography, such as gel filtration, ion exchange, hydrophobic interaction, reversed-phase, adsorption and affinity, have been used for the purification of NAs (**Table 3**).

Except in the case of gel filtration, the mode of operation of the chromatographic columns, whether small or large, is similar. Two possibilities exist:

1. Interaction of the target NA with the chromatographic support: column feed, binding of the target NA to the stationary phase, removal of impurities by washing and selective elution and, finally, elution of the target NA (**Figure 2**A).
2. No interaction of the target NA with the chromatographic support: column feed, collection of the target nucleic in the flow-through, binding of impurities to the stationary phase and, finally, column disposal or removal of the impurities by selective elution (**Figure 2**B).

A chromatographic column can also be used in conjunction with an enzyme process in order to improve the selectivity of the method. For instance, DNase (RNase) treatment of bound NAs will remove DNA (RNA), while leaving RNA (DNA) behind.

Purification by anion exchange takes advantage of the interaction between negatively charged phosphate groups in the DNA or RNA backbone and positively charged ligands on the surface of the particles that constitute the stationary phase. A salt gradient is usually used to displace the different NA species, which in principle should elute in order of increasing overall net charge, which in turn is a function of chain

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Table 3 Purification of nucleic acids by chromatography

Chromatography	*Basis of separation*	*Examples and applications*
Anion exchange	Charge, charge density	Plasmid purification Plasmid copy number analysis Fractionation of restriction fragments Separation of polymerase chain reaction products
Hydrophobic interaction	Hydrophobic interaction	Separation of dsDNA from ssDNA and RNA Fractionation of supercoiled and relaxed plasmids
Reversed-phase	Hydrophobic interaction	Separation of dsDNA from ssDNA and RNA Separation of supercoiled and relaxed plasmid Purification of oligonucleotides (chemical synthesis)
Adsorption	Selective adsorption	Capture of nucleic acids by silica (glass powder, diatomaceous earth) Separation of dsDNA from ssDNA by hydroxyapatite Separation of RNA from DNA with boronic acids
Affinity	Structure recognition	Triple helix formation Hybridization of poly(A)$^+$ tails to oligo(dT) ligands Plasmid purification with acridine dye ligands
Gel filtration	Size, shape	Buffer exchange Salt and oligonucleotide removal Fractionation of supercoiled and relaxed plasmids Removal of endotoxins

length. When column cartridges are used, the salt gradient is always a step for convenience, while in process applications linear gradients can improve selectivity.

Anion exchange chromatography can also be used as a nonsize-based NA purification tool. In some cases, base sequence and composition affect the elution pattern of NAs in anion exchangers. The shape and size of the molecules may also play an important role. This is the case, for example, in the purification of plasmid variants. In some anion exchangers, the more compact supercoiled plasmid forms, which have a higher charge density, elute later than the open circular forms, which have a lower overall charge density.

Since many of the NAs being isolated are normally very large molecules, their binding to most of the existent anion exchangers is likely to occur only at the surface. This constitutes an important capacity limitation, especially in large scale applications. Different types of stationary phases have been used for anion exchange chromatography of NAs. Typical examples include weak ligands such as diethylaminoethyl and dimethylamino coupled to silica, polymeric or composite (inorganic + polymeric) matrices and strong ligands such as quaternary amines coupled to polymeric matrices.

Hydrophobic interaction chromatography has not been described for the purification of NAs, but recent results indicate that matrices derivatized with mildly hydrophobic residues are able to separate double-stranded DNA from RNA and single-stranded DNA under nondenaturing conditions.

In reversed-phase chromatography, NAs are also retained by the hydrophobic interaction of the bases with the chromatographic resin, but here, the density of ligands is much higher. Separation of single- and double-stranded DNA has been accomplished in C_{18} columns and differentiation between DNA and RNA due to the effect of ribose and deoxyribose has also been reported. In reversed-phase chromatography, the binding is stronger than in hydrophobic interaction chromatography due to the higher ligand density. This requires elution to be carried out under more severe conditions, for instance, by using eluents with organic solvents, which can have a deleterious effect on the structure of the target molecule. For this reason, reversed-phase chromatography is more suited for the purification of smaller NAs that are less prone to denaturation. This is the case with synthetic oligonucleotides, used as primers or as blocking agents in antisense technologies, that are synthesized by solid-phase chemistry and purified by reversed-phase chromatography.

In adsorption chromatography, a stationary phase is used that selectively binds NAs in the presence of chaotropic salts, which remove water from hydrated molecules in solution, ensuring separation from complex biological mixtures. For instance, NAs adsorb to silica (glass) in the presence of sodium iodide or guanidinium hydrochloride, and to hydroxyapatite in the presence of urea. Polysaccharides and proteins do not adsorb and are removed by washing. Elution is performed using a low salt buffer. Hydroxyapatite further displays an ability to separate

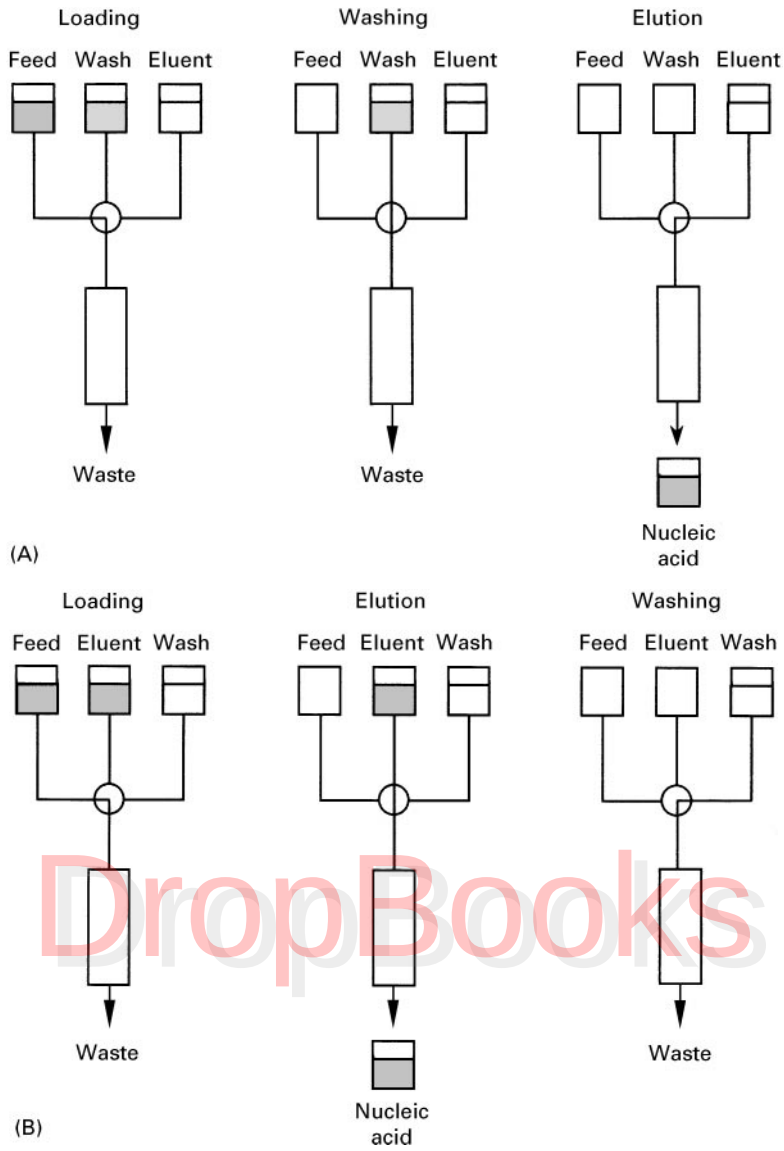

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Figure 2 Mode of operation of chromatographic columns in nucleic acid purification: (A) interaction of the target nucleic acid with the chromatographic support; (B) no interaction of the target nucleic acid with the chromatographic support.

single-stranded NAs that bind less tightly from double-stranded NAs.

A method for the selective adsorption of RNA in the presence of DNA employs immobilized boronic acid derivatives which, above their pK_a values, form cyclic complexes with vicinal diols. Since the deoxyribose sugars lack the vicinal diol of ribose sugars in RNA, DNA is poorly adsorbed and can be washed away easily.

Affinity chromatography is based on the recognition of a particular structure in the target NA molecule by an immobilized ligand. In triple-helix affinity chromatography, the formation of triple helices between oligonucleotides linked to a chromatographic matrix and duplex sequences present on dsDNA is explored. The affinity of poly(A)$^+$ tails of mRNA to oligo(dT) probes has also been explored as a way of capturing and purifying mRNA. The combination of affinity ligands with magnetic particles is another recent development that avoids the use of packed columns. It allows the binding of the target molecule directly from solution, with the particle complex being recovered with a magnet. The high selectivity of affinity chromatography makes it a powerful tool for the one-step purification of NAs. However, since each ligand targets a specific base sequence, the versatility of the technique is low and the associated cost high.

Size exclusion or gel filtration chromatography has been used to fractionate NA molecules on the basis of

their relative size. By selecting a gel with an appropriate selectivity for the size range in question it is possible, for example, to isolate supercoiled plasmid DNA from microbial contaminants such as genomic DNA, RNA, proteins and endotoxins. Another feature of size exclusion chromatography, often explored when purifying NAs, is the possibility of exchanging buffers and removing salts, nucleotides, excess primers and other small molecules. This type of column is commercially available in the cartridge format for the clean-up of NA solutions.

Quality Control of Final Nucleic Acid

The final NA quality criteria will vary with the NA inherent complexity, its intended use and the method and complexity of manufacture. The researcher and/or producer will select several quality control tests that depend on their own application. NAs can be quantitatively and qualitatively analysed by a number of chemical, biochemical and physical assays. Some quality controls of NA preparations are summarized in **Table 4**.

A method used widely to estimate the amount of RNA or DNA is spectrophotometric analysis. The absorbance at 260 nm is relatively accurate (depending on base composition) and reproducible when applied to purified samples without significant amounts of contaminants (e.g. proteins, other NAs, phenol), and to moderately diluted or concentrated preparations. Spectrophotometric scans between 220 and 320 nm are also used to detect salt and organic contaminations. The purity of NAs based on the absorbance ratio 260 nm/280 nm is commonly used. A 260 nm/280 nm ratio between 1.8 and 2.0 is usually considered to be a good estimation of purity. This method was initially developed to quantify the contaminating NAs in protein preparations. For this reason, it often fails when used for NA purity assessment. Therefore, when a high level of purity is critical, care should be taken while using this method, and it should be complemented by other purity analysis. HPLC, capillary electrophoresis or methods using specific fluorescent dyes, quantitative DNA or RNA hybridization and quantitative polymerase chain reaction are preferable.

Table 4 Quality control tests for DNA or RNA preparations

Test	*Method*
Appearance	Visual inspection
Identity	Restriction enzyme analysis
	Gel and capillary electrophoresis
	Sequencing
Concentration	Spectrophotometry A260 nm
	HPLC
	Gel and capillary electrophoresis
Other nucleic acids	Gel and capillary electrophoresis
	DNA or RNA hybridization
	HPLC
Proteins	Colorimetric assay (e.g. BCA)
	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
	Immunoassays
Polysaccharides	Specific assays (e.g. HPLC, enzymatic
Lipopolysaccharides	assays, LAL)
Nucleases	Nuclease-specific assays
Sterility	Cytopathic effects
	Reverse transcriptase assay
	Electron microscopy
	Bioburden assay

Conclusions

Isolation/purification of nucleic acids is becoming more and more important with the emergence of areas such as genomics, gene therapy and DNA vaccination and the growing importance of clinical diagnostics and forensics. The general strategies and technologies commonly used in the purification and isolation of NAs have been reviewed in this article. Critical issues and bottlenecks that still hamper the purification of nucleic acids have also been highlighted. Although the future will certainly bring more efficient isolation/purification methodologies, designed for high-throughput and automated preparation/analysis, the core technologies and strategies described here will most likely retain their importance.

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ESSENTIAL GUIDES FOR ISOLATION/ PURIFICATION OF POLYSACCHARIDES

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Introduction

The isolation of polysaccharides from biological sources represents an important source of these valuable materials. Biomass such as cereal straws and grasses, are an enormous underutilized energy resource as raw materials in the production of paper, panel products, chemicals and other industrial products. On a dry-weight basis, the straws and grasses contain 65–85% of polysaccharides, with hemicelluloses ranked second to cellulose in abundance; however, it must be noted that the chemical content of the hemicellulose, with respect to saccharide ratios changes with plant growth and maturity (**Table 1**).

At the present time, there is widespread interest in the use of hemicelluloses, particularly arabinoxylan-rich hemicelluloses as precursors in food gums. More recently, water-soluble xylans from corn cobs have shown biological activity as immuno-modulating compounds. Other potential industrial applications of hemicelluloses are to be found in the fields of adhesives, thickeners in foods, stabilizers, biodegradable film formers and emulsifiers. They can also be easily converted to primary chemicals such as xylose, xylitol, furfural, hydroxymethylfurfural and levulinic acid.

Hemicelluloses, however, are the most complex components in the cell wall of straws and grasses. They form hydrogen bonds with cellulose, covalent bonds (mainly α-benzyl ether linkages) with lignins and ester linkages with acetyl units and hydroxycinnamic acids. To investigate the potential utilizations of polysaccharides from straws and grasses, a thorough study of the isolation procedures is necessary. Details of the method are reviewed as follows.

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Cell Wall Preparation

The straw or grass is cut into 1–2-cm lengths, air-dried, and ground to pass through a 0.5–0.8-mm screen. The ground sample is then further dried in a cabinet oven with air circulation at 50–60°C for 12–16 h. Dried material is dewaxed by refluxing with toluene–EtOH (2 : 1, v/v) or defatted with chloroform–methanol (2 : 1, v/v) for 6 h in a Soxhlet apparatus. The dewaxed sample is treated with α-amylase to degrade the starch or extracted with phenol–acetic

Table 1 Chemical composition of agricultural residues (per cent dry matter)

Species	*Water-solubles*	*Cellulose*	*Hemicelluloses*	*Lignin*	*Extract*	*Ash*
Wheat straw	4.7	38.6	32.6	14.1	1.7	5.9
Rice straw	6.1	36.5	27.7	12.3	3.8	13.3
Rye straw	4.1	37.9	32.8	17.6	2.0	3.0
Barley straw	6.8	34.8	27.9	14.6	1.9	5.7
Oat straw	4.6	38.5	31.7	16.8	2.2	6.1
Maize stems	5.6	38.5	28.0	15.0	3.6	4.2
Corn cobs	4.2	43.2	31.8	14.6	3.9	2.2
Esparto	6.1	35.8	28.7	17.8	3.4	6.5
Sugar beet pulp	(pectin 27.1) 5.9	18.4	14.8	5.9	1.4	3.7
Bagasse	4.0	39.2	28.7	19.4	1.6	5.1
Oil palm fibre	5.0	40.2	32.1	18.7	0.5	3.4
Abaca fibre	3.7	60.4	20.8	12.4	0.8	2.5